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Survey of *Giardia* and *Cryptosporidium* in lemurs from the Ranomafana National Park, Madagascar

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ABSTRACT: We detected *Cryptosporidium* sp. by direct immunofluorescence in fecal samples from greater bamboo lemurs (*Prolemur simus*) and eastern rufous mouse lemurs (*Microcebus rufus*) inhabiting the Ranomafana National Park, Madagascar. This is the first report of an occurrence of these potentially zoonotic parasites in free-ranging lemurs in the rain forest of Madagascar.

Expansion of human settlements in the vicinity of pristine natural areas is increasing the risk of disease emergence and transmission to wildlife populations (Daszak et al., 2000; Gillespie et al., 2008). Among the potentially emerging threats, *Giardia* spp. and *Cryptosporidium* spp. are two potentially zoonotic protozoan parasites that can infect a wide range of species, including humans, and domestic and wild animals. These parasites, which are highly prevalent in developing countries, are transmitted by a fecal-oral cycle, either directly or via contaminated material, water, or food (Monis and Thompson, 2003). Therefore, these two parasitic diseases represent a potential risk of anthroponosis to naïve wildlife populations (Graczyk et al., 2002).

Giardia spp. are ubiquitous, flagellated protozoan parasites causing significant disease in a variety of avian, mammalian, and reptilian species (Adam, 2001). They are among the most common human intestinal parasites, and they are frequent enteric parasites of domestic animals, including livestock, dogs, and cats. *Giardia* spp. are also commonly isolated in captive wildlife and are sometimes associated with clinical signs such as diarrhea (Levecke et al., 2007). *Cryptosporidium* spp. are coccidian, protozoan parasites that can cause gastrointestinal disease in a wide range of wild and domestic animals and humans (Appelbee et al., 2005). They have been reported in several species of free-ranging and captive primates, in which they have caused significant disease. A factor of note, *Cryptosporidium* spp. are a major cause of diarrhea and mortality in captive lemurs (Charles-Smith et al., 2010).

Despite numerous studies investigating lemur health in diverse, protected areas (Junge et al., 2008), *Cryptosporidium* spp. have only been reported or suspected in free-ranging, ring-tailed lemurs from the dry forest of southern Madagascar (Villers et al., 2008). Neither *Giardia* nor *Cryptosporidium* has been previously identified in lemurs from Ranomafana National Park (RNP), Madagascar (Wright et al., 2009). We assessed the presence of *Giardia* and *Cryptosporidium* parasites in lemurs from the RNP, Madagascar.

The RNP encompasses 43,500 ha of continuous, midaltitude rain forest located 47°18′40 to 47°37′E and 21°2′ to 21°25′S and comprises two trail systems, one in disturbed habitat (Talatakely trail system, TTS) and one in a pristine portion of rain

TABLE 1. Protozoan parasites detected in fecal samples of free-ranging lemurs from the Ranomafana National Park, Madagascar, using an immunofluorescence test, July 2011.

Species	*Cryptosporidium* (positive/collected)		*Giardia* (positive/collected)	
	TTS[a]	VALO[b]	TTS	VALO
Milne Edwards' sifaka (*Propithecus edwardsi*)	0/6	0/9	0/6	0/9
Red-bellied lemur (*Eulemur rubriventer*)	0/5	0/10	0/5	0/10
Greater bamboo lemur (*Prolemur simus*)	2/4	—[c]	0/4	—
Eastern rufous mouse lemur (*Microcebus rufus*)	2/4	—	0/4	—
Total	4/19	0/19	0/19	0/19

[a] Talatakely trail system.

[b] Valohoaka.

[c] No sample collected.

forest (Valohoaka). The TTS was selectively logged in the mid-1980s and is now heavily visited by tourists. Valohoaka has minimal disturbance and low tourist visitation (Wright et al., 2012).

Fresh fecal samples were collected noninvasively during the dry season (July 2011) from four species of free-ranging lemurs inhabiting the RNP: Milne Edwards' sifaka (*Propithecus edwardsi*), greater bamboo lemur (*Prolemur simus*), red-bellied lemur (*Eulemur rubriventer*), and eastern rufous mouse lemur (*Microcebus rufus*). Lemurs were tracked and identified using telemetric devices placed during previous projects, and fecal specimens were collected after observation of defecation, avoiding collection of multiple samples from the same animal. Fecal samples were examined macroscopically for mucus or parasites, and the consistency of the fecal sample was noted upon collection. Two to 3 grams of fecal material were immediately fixed in 10% buffered formalin and stored at room temperature until analysis. Age, sex, and location of the lemur were recorded. The presence of clinical signs of diseases was assessed by examining the mentation (alert or lethargic) and responsiveness of the individual, as well as the presence of diarrhea. Fecal samples were examined for *Cryptosporidium* spp. and *Giardia* spp. by direct immunofluorescence with a commercial kit (Meridian Merifluor Giardia/Crypto®, Meridian Diagnostics, Inc., Cincinnati, Ohio, USA) as described by Olson et al. (1997).

All 38 samples collected had normal consistency, and no sign of diarrhea was noted. All samples examined were negative for *Giardia*; however, *Cryptosporidium* oocysts were detected in 4 (10.5%) of the 38 fecal specimens. Two greater bamboo lemurs and two eastern rufous mouse lemurs from the TTS were positive by immunofluorescence. Oocysts were ovoid, 4.5–5.1 × 4.5–4.9 μm, and morphologically indistinguishable from *Cryptosporidium parvum*, *Cryptosporidium hominis*, or *Cryptosporidium canis* (Monis and Thompson, 2003). Table 1 shows the number of fecal samples collected and the results of the parasitologic analysis.

Failure to detect *Giardia* in the samples suggests the absence of *Giardia* in the parasitic communities of lemurs, the seasonality of *Giardia* cyst excretion (Adam, 2001), or a lack of sensitivity due to small sample size. To our knowledge, this is the first report of *Cryptosporidium* in the eastern rufous mouse lemur and the critically endangered greater bamboo lemur. No signs of disease were noted in the lemurs sampled. In most animal species, and in lemurs in particular, *Cryptosporidium* usually affects younger or immunocompromised individuals, causing diarrhea or even death (Charles-Smith et al., 2010). Our failure to observe overt clinical signs of cryptosporidiosis in positive individuals may be because we only sampled adult lemurs.

We also report for the first time the occurrence of these potentially zoonotic or anthroponotic (transmitted from humans to animals) parasites in lemurs from the rain forest of Madagascar. Presence of *Cryptosporidium* in wild nonhuman primates has been argued to indicate increased contact with humans or domestic livestock (Nizeyi et al., 2002a, 2002b). Consequently, the presence of these parasites in free-ranging lemurs inhabiting the RNP may be due to parasite spillover directly from humans, or from domestic animals or introduced rodents.

Alternatively, the *Cryptosporidium* sp. found in lemurs at RNP may be an undescribed species with a sylvatic cycle. Morphologic identification of *Cryptosporidium* does not yield conclusions on the zoonotic or anthroponotic potential of these parasites (Monis and Thompson, 2003). Molecular characterization is needed to determine the species of *Cryptosporidium* infecting lemurs at the RNP and to better understand the ecology of this parasite. Evaluation of risk factors linked to *Cryptosporidium* infection in lemurs and assessment of the possible impact of these parasites on lemur health and survival in the wild are also needed.

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